



**University of
Zurich^{UZH}**

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2012

Light perception: more than meets the eyes

Mueller, Kaspar P ; Neuhauss, Stephan C F

Abstract: Larval zebrafish lacking eyes and pineal organ show elevated activity levels and undirected light-seeking behaviour upon loss of illumination. This behaviour, termed dark photokinesis, is mediated by hypothalamic deep brain photoreceptors expressing melanopsin.

DOI: <https://doi.org/10.1016/j.cub.2012.08.038>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-67124>

Journal Article

Accepted Version

Originally published at:

Mueller, Kaspar P; Neuhauss, Stephan C F (2012). Light perception: more than meets the eyes. *Current Biology*, 22(21):R912-R914.

DOI: <https://doi.org/10.1016/j.cub.2012.08.038>

Light Perception: More than Meets the Eyes

Larval zebrafish lacking eyes and pineal organ show elevated activity levels and undirected light seeking behavior upon loss of illumination. This behaviour, termed dark photokinesis, is mediated by hypothalamic deep brain photoreceptors expressing melanopsin.

The proverbial man on the street would unhesitatingly agree that the eyes are the major site for light perception in vertebrates. Indeed, they are the only structure responsible for image-forming vision and control most observable visual behaviours. However, less appreciated are additional light sensitive structures outside of the eyes, that are common at least in nonmammalian vertebrates (Fig. 1). The best known example is the pineal complex, which, in nonmammalian vertebrates, is directly light sensitive and governs circadian entrainment of physiological functions, including linking of sleep-wake cycles to light and dark periods [1, 2]. In addition to the pineal complex, dermal melanophores of nonmammalian vertebrates contain photoreceptors which control skin colour depending on ambient light levels [3]. Already at the beginning of the 20th century, behavioural experiments in fish and birds suggested the existence of deep brain photoreceptors located in the hypothalamus as a further site for light perception in nonmammalian vertebrates [4, 5].

Considering the mostly light-impermeable skull of vertebrates and the location of the hypothalamus deep within the brain, this is certainly not the first place to look for light

sensitive structures. Consequently, not only the existence of deep brain photoreceptors, but also their exact location within the brain, their identity, as well as the light sensing pigments involved, have been a matter of debate for over a century. Some of these questions have now definitively been answered in a series of elegant experiments, combining behavioral experiments and genetically targeted ablation of cells [6]. In this issue, Fernandes *et al.* [6] show that zebrafish larvae lacking the well-established light sensitive structures – i.e. eyes and pineal organ – still react to a sudden loss of illumination with an elevated locomotor activity and an undirected light-seeking behavior. They further demonstrate that this behavior is triggered by a group of neurons in the preoptic area of the hypothalamus and that the light sensing pigment responsible for mediating this reaction is melanopsin.

The authors of this study observed that zebrafish larvae exposed to a sudden loss of illumination, and, at the same time, presented a target light in one part of their swimming arena, display some distinct behaviors: First, they perform an orientation maneuver (termed O-turns) towards the target light. Second, a series of slower turns (termed R-turns) brings them closer to the target light, where they finally aggregate. Surprisingly, this behavior is not completely abolished if the larval eyes are surgically removed. Such enucleated larvae fail to perform O-bends, and hence fail to orient themselves towards the target light, but still display an increased rate of R-turns which will ultimately let them aggregate in the illuminated portion of the arena. This aggregation is achieved in a stochastic manner: Upon loss of illumination, blinded zebrafish larvae become hyperactive until they reach the illuminated site by chance. At this point, their activity

decreases, thereby “trapping” the larvae in a bright zone. A robust measure for the hyperactivity induced by a loss of illumination (dark photokinesis) is the visual motor response assay, where gross locomotor activity of many larvae in relation to the illumination can be measured in parallel. Since dark photokinesis can be triggered in enucleated larvae, the pineal organ – given its known light sensitivity in teleosts – suggests itself as the relevant structure and thus was the first organ the authors tested for its involvement in the observed reaction. Surprisingly, dark photokinesis could not be abolished by a genetically targeted ablation of the pineal complex, neither in intact, nor in enucleated larvae which accordingly lacked both eyes and pineal organ. The authors therefore searched for visual pigments that are expressed in neither the eyes nor the pineal gland, as potentially light receptors mediating photokinesis. They considered two promising candidates: teleost multiple tissue opsin a (*tmtopsa*) and the invertebrate-like opsin melanopsin 4a (*opn4a*), both expressed in a domain in the hypothalamus defined by the orthopedia (*otpa*) transcription factor. Indeed, dark photokinesis was absent in *otpa* mutant fish, a finding which was confirmed by transgenic ablation of *otpa*-expressing neurons. *tmtopsa* could be ruled out as a candidate, since it was not reduced in *otpa* mutants; the authors thus concluded that melanopsin expressed in hypothalamic neurons must be the light absorbing pigment responsible for triggering dark photokinesis. Their conclusions was confirmed by their demonstration that overexpression of *opn4a* within the *otp*-domain leads to an increase of dark photokinesis in enucleated larvae. By further ablation experiments, an involvement of *opn4a*-expressing dopaminergic cells of the hypothalamus could be excluded, narrowing the region of interest to the preoptic area of the hypothalamus.

As earlier mentioned, deep brain photoreceptors in the hypothalamus of nonmammalian vertebrates were already suggested at the beginning of the 20th century. It was none other than the celebrated Austrian ethologist and Nobel prize laureate Karl von Frisch (1886-1982), who observed that Eurasian minnows (*Phoxinus phoxinus*), still display colour changes of their skin in response to light after removal of the eyes and the pineal organ [5]. Based on these findings, von Frisch proposed the existence of some ‘deep-diencephalic photoreceptors’, a hypothesis corroborated by experimental findings in the European eel (*Anguilla anguilla*), showing photonegative behavior in the absence of eyes and pineal organ [7]. Evidence for the existence of photoreceptive cells within the hypothalamus was also collected in birds, by showing that in blinded ducks and sparrows, testicular growth can be induced by illuminating the hypothalamus [4, 8-10]. Since these early studies, the existence of so-called deep brain photoreceptors has been demonstrated in a number of nonmammalian vertebrate species and, more recently, also in neonatal rats and mice [11, 12]. Deep brain photoreceptors have been mainly implicated in the regulation of circadian and circannual physiology, e.g. in reptiles for the entrainment of circadian rhythms of locomotor activity to light-dark cycles [13-15] and in the control of seasonal breeding or migratory behavior in birds [16, 17], but also in the control of light-seeking or light-avoiding behavior in reptiles and neonatal mammals [7, 11, 12]. Even though behavioral evidence for the existence of deep brain photoreceptors was collected over the years in a number of species, their exact location and embodied photopigment could not be determined so far. We had to wait for over a century after the seminal work of von Frisch and his suggestion of deep brain photoreceptors, until the ingenious

combination of exploiting the genetic and behavioral strength of the zebrafish model provided evidence for both the location and the nature of the deep brain photoreceptors. Fernandes *et al.* for the first time assigned the location of these receptors to a group of neurons in the preoptic hypothalamus and unequivocally determined that melanopsin mediates light perception. At the same time, the authors provide an explanation for the thus far puzzling observation that zebrafish larvae at 28 hours post fertilization already react to the offset of light by increasing their locomotor activity – notably at a developmental stage even before the first ganglion cell axons exit the retina and hence the eyes are unerringly nonfunctional [18].

These are indeed exciting times for ethologists to see the field evolving into true neuroethology with the opportunity to unravel a connection between behavior and well defined neurons in the brain.

References:

1. Bertolucci, C., and Foa, A. (2004). Extraocular photoreception and circadian entrainment in nonmammalian vertebrates. *Chronobiol. Int.* 21, 501-519.
2. Vigh, B., Manzano, M.J., Zádori, A., Frank, C.L., Lukáts, A., Röhlich, P., Szél, A., and Dávid, C. (2002). Nonvisual photoreceptors of the deep brain, pineal organs and retina. *Histol. Histopathol.* 17, 555-590.
3. Weber, W. (1983). Photosensitivity of Chromatophores. *Am. Zool.* 23, 495-506.

4. Benoit, J. (1935). Stimulation par la lumière artificielle du développement testiculaire chez des canards aveuglés par énucléation des globes oculaires. *Comp. Rend. Soc. Biol.* *120*, 136-139.
5. von Frisch, K. (1911). Beiträge zur Physiologie der Pigmentzellen in der Fischhaut. *Pflug. Arch. Eur. J. Physiol.* *138*, 319-387.
6. Fernandes, A.M., Fero, K., Arrenberg, A.B., Bergeron, S.A., Driever, W., and Burgess, H.A. (2012). Deep brain photoreceptors control light seeking behavior in zebrafish larvae. *Curr. Biol.* *This Issue*.
7. van Veen, T., Hartwig, H.G., and Muller, K. (1976). Light-dependent motor activity and photonegative behavior in the eel (*Anguilla anguilla* L.). *J. Comp. Physiol.* *111*, 209-219.
8. Benoit, J. (1964). The role of the eye and of the hypothalamus in the photostimulation of gonads in the duck. *Ann. N.Y. Acad. Sci.* *117*, 204-215.
9. Menaker, M., and Keatts, H. (1968). Extraretinal light perception in the sparrow. II. Photoperiodic stimulation of testis growth. *Proc. Natl. Acad. Sci. USA* *60*, 146-151.
10. Yokoyama, K., Oksche, A., Darden, T.R., and Farner, D.S. (1978). The sites of encephalic photoreception in photoperiodic induction of the growth of the testes in the White-crowned Sparrow, *Zonotrichia leucophrys gambelii*. *Cell Tiss. Res.* *189*, 441-467.
11. Johnson, J., Wu, V., Donovan, M., Majumdar, S., Renteria, R.C., Porco, T., Van Gelder, R.N., and Copenhagen, D.R. (2010). Melanopsin-dependent light avoidance in neonatal mice. *Proc. Natl. Acad. Sci. USA* *107*, 17374-17378.

12. Routtenberg, A., Strop, M., and Jerdan, J. (1978). Response of the infant rat to light prior to eyelid opening: mediation by the superior colliculus. *Dev. Psychobiol.* *11*, 469-478.
13. Foà, A., Flamini, M., Innocenti, A., Minutini, L., and Monteforti, G. (1993). The role of extraretinal photoreception in the circadian system of the ruin lizard *Podarcis Sicula*. *Comp. Biochem. Physiol.* *105*, 223-230.
14. Pasqualetti, M., Bertolucci, C., Ori, M., Innocenti, A., Magnone, M.C., De Grip, W.J., Nardi, I., and Foa, A. (2003). Identification of circadian brain photoreceptors mediating photic entrainment of behavioural rhythms in lizards. *Eur. J. Neurosci.* *18*, 364-372.
15. Underwood, H., and Menaker, M. (1976). Extraretinal photoreception in lizards. *Photochem. Photobiol.* *23*, 227-243.
16. Foster, R.G., and Follett, B.K. (1985). The involvement of a rhodopsin-like photopigment in the photoperiodic response of the Japanese quail. *J. Comp. Physiol. A* *157*, 519-528.
17. Yokoyama, K., and Farner, D.S. (1978). Induction of Zugunruhe by photostimulation of encephalic receptors in white-crowned sparrows. *Science* *201*, 76-79.
18. Kokel, D., Bryan, J., Laggner, C., White, R., Cheung, C.Y., Mateus, R., Healey, D., Kim, S., Werdich, A.A., Haggarty, S.J., et al. (2010). Rapid behavior-based identification of neuroactive small molecules in the zebrafish. *Nat. Chem. Biol.* *6*, 231-237.

19. Peirson, S.N., Halford, S., and Foster, R.G. (2009). The evolution of irradiance detection: melanopsin and the non-visual opsins. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 2849-2865.

Affiliations:

Kaspar P. Mueller and Stephan C.F. Neuhauss

University of Zurich, Institute of Molecular Life Sciences; Neuroscience Center Zurich and Center for Integrative Human Physiology, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

kaspar.mueller@imls.uzh.ch

stephan.neuhauss@imls.uzh.ch

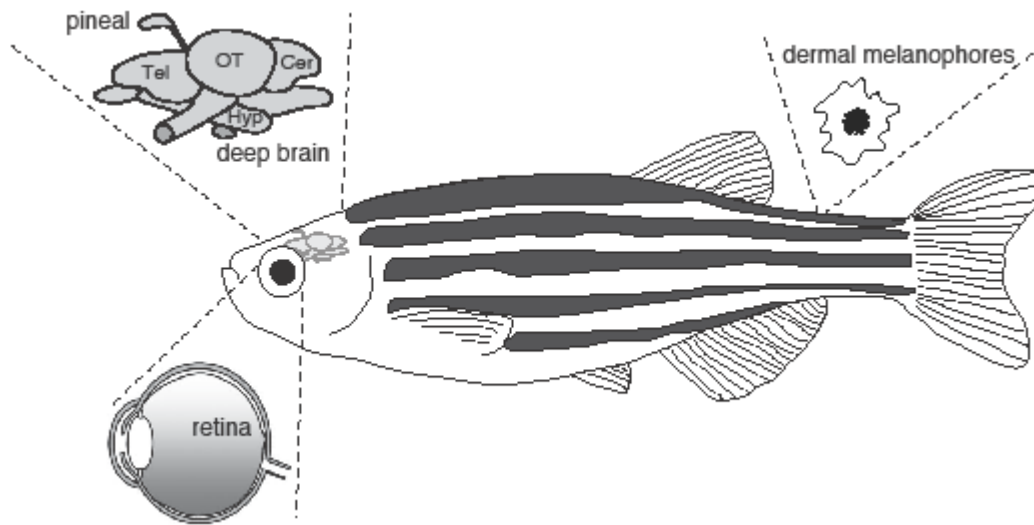


Figure 1. Photosensitive structures in nonmammalian vertebrates

Apart from the retina – the light sensitive tissue in the back of a vertebrate eye – photosensitive structures and tissues in nonmammalian vertebrates include the pineal complex, deep brain photoreceptors in the hypothalamus as well as dermal melanophores. Tel: Telencephalon; OT: Optic Tectum; Cer: Cerebellum; Hyp: Hypothalamus. Modified after [19].